

**Review Article****FACTORS INFLUENCING HEAVY METAL REMOVAL BY MICROALGAE-A REVIEW****SIBI G.\*****Head of the Department, Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India**  
Email: gsibii@gmail.com*Received: 04 Sep 2019 Revised and Accepted: 10 Oct 2019***ABSTRACT**

The build-up of heavy metals in soils and waters continues to create serious global health concerns, as these metals cannot be degraded into non-toxic forms, but persist in the ecosystem. It is essential to remove or reduce heavy metal contamination in order to prevent or reduce contaminating the environment. Bioremediation utilizes inherent biological mechanisms to eliminate hazardous contaminants using microorganisms and plants. Algae have been applied to heavy metal removal because of their high sorption capacity. This review addresses the different factors influencing heavy metal removal by algae and its corresponding application in bioremediation. Based on the earlier reports, microalgae have the potential to become an effective and economical bio sorbent for the bioremediation of heavy metal. Further, the development of technology for simultaneous detection and removal of toxic metals using microalgae will assist in bioremediation of toxic metal from contaminated sites and from industrial effluents.

**Keywords:** Heavy metals, Bioremediation, Microcode, Pollution, Abiotic factors

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**INTRODUCTION**

Metal represents an integral constituent of the ecological system whose availability and concentration depends on its cycling in various geological and biological processes taking place in that ecosystem [1]. Through geochemical processes such as weathering of rocks and volcanic eruptions are considered as the natural reason behind the occurrence of metal, a significant amount of metal coming into the environment comes from anthropogenic activities. Heavy metals are defined as elements of metallic properties having the atomic number 420 [2, 3]. Increasing urbanization and industrialization has made heavy metal reach alarming toxic levels in the environment. Human activities that lead to an enrichment of metals and metalloids in aquatic systems include discharges of untreated effluents from mining, refining, electroplating, chemical and metallurgical industries, while biological processes include geological weathering [4-7].

The most common heavy metal contaminants include Cd, Cr, Cu, Hg, Pb, and Zn. Heavy metals are key environmental pollutants in regions with high anthropogenic pressure, and their presence, even in traces, can cause serious problems with several organisms and humans [8]. Due to their mobility in aquatic ecosystems and, their toxicity to higher life forms, heavy metals in surface and groundwater supplies has been prioritized as the major inorganic contaminants in the environment [9]. Conventional Physico-chemical methods for heavy metal removal such as ion exchange, chemical precipitation or oxidation and solvent extraction, often prove ineffective or require high energy input, capital investment and operational costs [10-12]. It is essential for a remediation technology to be applicable, economic/affordable and consistent. Several types of both living and non-living microorganisms including yeast, bacteria, algae, and fungi, have shown high binding and uptake capacities with regard to metals and metalloids [5, 13, 14] suggesting the potential for developing biological technologies for metal removal from aqueous media.

This review focused on bioremoval of heavy metals using microalgae to reduce the heavy metal contaminants in the environment. A systematic search was carried out in Pub Med, Scopus and Web of Sciences using a combination of Boolean operators. Peer-reviewed papers in English on the keyword heavy metal removal by microalgae were retrieved and evaluated based on titles and abstracts. The retrieved papers were managed using Mendeley and the data were consolidated.

Heavy metal adversely affects microbial growth by inhibiting enzyme activity and bringing about change in nucleic acid and protein conformations. While prokaryotes are known to possess enough metal defense strategies, as they are the foremost biota that has evolved in the surrounding of metal, likewise, the eukaryotic microbial system has also been reported to have enough strategies to survive in metal stress. Depending on the surrounding conditions, microorganisms have developed numerous extracellular and intracellular defense mechanisms that help them cope with the toxic effect of metals [15]. While extracellular mechanisms prevent the entry of metal into the cell interior, intracellular mechanisms basically prevent the cell from the saddle of toxic metals that have been taken up in excess by the cell [16].

Metal ions are taken up in a two-stage adsorption and bioaccumulation process. At first, the metal ions are passively adsorbed on the cell surface (of both living and nonliving biomass) just in a few seconds or minutes; then the ions are carried slowly inside the cell membrane and are accumulated intracellular. Bioaccumulation occurs only in living cells, involving transport of metal ions across the cell membrane barrier and subsequent binding to cytoplasmic proteins or polysaccharides, or to specific cellular compartments such as vacuoles or polyphosphate bodies. The two above described processes, involving both adsorption and bioaccumulation by living and/or dead microorganisms, can be defined as 'biosorption'. Algae have high sorption capacities and strong metal ion sorption selectivity [17-20]. Some microalgae and cyanobacteria are metal stress-tolerant and possess high resistance towards metal toxicity, large surface area and high binding affinity [21].

Interaction of algae with toxic metal severely affects their physiological and enzymatic activity ultimately leading to their death. Algae, in turn, have promoted efficient defense mechanisms that alleviate the toxic effects generated by the metal ions and favor their survival at elevated concentrations of these metals. They employ both extracellular and intracellular modes for combating metal toxicity. Microalgae have been recorded to precipitate and bio accumulate the entrapped metal ions in different cellular organelles as a means to survive heavy metal stress. The large surface area to volume ratio and high-affinity functional groups on the cell surface of microalgae makes them susceptible for uptake and hence serves as proficient agents for metal storage systems [22]. The metal accumulation capacity of algal biomass is to comparable or

sometimes higher than chemical sorbents. Therefore, algal biomass may be used as an economically feasible and efficient alternative to the existing physicochemical methods of metal removal [23].

Distinct binding groups, such as OH-, SH-, COO-, PO<sub>4</sub><sup>3-</sup>, NO<sup>3-</sup>, RNH<sup>2-</sup>, RS- and RO-promote the metal ion adsorption. These binding groups are attended by the cell surface, in the cytoplasm, and especially vacuoles. Since metal ions in water are usually in the cationic form, they are adsorbed onto the cell surface. If the mechanism of metal ion bioremediation is the uptake of ions by algal cells, cytosolic proteins mediate the transfer of metal ions into the cells [24]. The toxic level of heavy metal ions in variant algal species can be highly strained specific, which consequently determines the potential remediation capacity using a distinctive algal strain. In other words, a heavy metal ion may exhibit a selective interaction with one definite algal strain, in addition to differences between similar species. Algae cell walls are the first barrier against the biosorption of heavy metal ions. Polysaccharides and proteins present in algae cell walls have the greatest metal-binding sites [25]. Due to the different distribution and abundance of cell wall compositions in different algal strains, the capacity of metal ions biosorption by the variant algal strains will vary.

Various studies have shown that microalgae is capable of binding heavy metals like copper, zinc, nickel, cadmium, and lead. The metal sorption depends on biomass concentration, pH, temperature and availability of the heavy metal. The review addresses the different factors influencing heavy metal removal by algae and its corresponding application in bioremediation.

### Factors influencing heavy metal ion biosorption

#### Biomass concentration

The effect of biomass concentration on metal removal was investigated by many authors. Increasing the biomass concentration has apparently improved the metal absorption due to the higher number of available metal-binding sites [26]. At the same time, decreased metal removal is often reported at very high biomass levels due to partial aggregation of biomass, as well as, by a decrease in the average distance between available adsorption sites. Bishnoi *et al.* [27] reported that copper uptake decreases when the biosorbent concentration is increased (40.5 g l<sup>-1</sup>). Similarly, a marked reduction in lead uptake by *Spirulina maxima* was reported when biomass concentration increased from 0.1 to 20 g l<sup>-1</sup> [28]. Romera *et al.* [29] reported that maximum biosorption efficiency could be obtained at the lowest biomass concentration.

#### pH

Most of the studies have shown that the sorption of metal ions in batch as well as in a continuous system, is a function of pH of the solution. pH can affect the solubility and toxicity of heavy metals in the water; it is perhaps the most important parameter influencing metal adsorption by microalgal biomass [30-32]. It affects the speciation of metals in solution and algal tolerance [30]; particularly, it influences both, cell surface metal-binding sites, and, metal chemistry in water. Dependence of metal ion uptake on pH is related to the metal ion complexation chemistry in solution, and behavior of many different functional groups presents at the surface of algal cells as well as to complex formation constants [11, 33, 34].

Peterson *et al.* [35] proved of pH-dependent metal toxicity in their report on algae. Since a majority of the metal-binding groups of algae are acidic (e. g., carboxyl), their availability is pH-dependent. These groups generate a negatively charged surface at acidic pH, and electrostatic interactions between cationic species and the cell surface are responsible for metal biosorption. The sorption of Cr (VI) and Cd on *Padina* sp. and *Sargassum* sp. [36], and Cesium sorption on *Padina australis* [37] was optimal at pH 2. Yu *et al.*, [38] found very little sorption of Cu by *Durvillaea potatorum* at pH below 2. It is well known that high amounts of uranium (VI) are bound by *Chlorella vulgaris* in the pH range from 3 to 6 [39]. Han *et al.*, [33] investigated the Cr (III) uptake by *Chlorella minima* biomass and found that biosorption capacity in pH 3, 4 and 4.5, was 14.17, 28.72 and 41.12 mg Cr (III)/g dried algae, respectively. Similar research by Gupta and Rastogi [11] on the uptake of Pb (II) by *Spirogyra* sp.

biomass showed that biosorption of Pb (II) at the pH<3, is very low. When the pH increased in the range of 3-5, an increase in lead ions sorption was observed, with the maximum amount of sorbed ions being 140 mg/g at pH 5. Species of *Desmodesmus* and *Heterochlorella* have the potential to withstand heavy metals such as Cu, Fe, Mn and Zn at an acidic pH of 3.5. These microalgae grew well, effecting predominant intracellular accumulation as well as removal of the selected metals [40]. Yan and Viraraghavan [41] further suggested that at acidic pH, H<sub>3</sub>O<sup>+</sup> ions prevent metal adsorption as they cover the binding sites on the cell walls, while at alkaline pH, the functional groups are freely permitting the binding of metal ions.

#### Initial metal ion concentration

Heavy-metal ion removal by algal biomass depends largely on the initial concentration of metal ions in the solution phase. Biosorption initially increases as the initial concentration of metal ion increases. Metal sorption initially increases with an increase in metal concentration in the solution and then becoming saturated after a certain concentration of metal [42-44]. Monteiro *et al.*, [45] reported a 5-fold increase in initial concentrations of Zn (II) (from 10 to 50 ppm) boosted the metal ion sorption from 19 to 209.6 mg Zn (II)/g dry biomass of *Scenedesmus obliquus*. In other words, the higher metal ion concentrations the lower the efficiency [46].

Microalgal growth showed to decrease at increasing metal concentrations, but promising metal removal efficiency was recorded up to 43% and 39% for Cu by *Desmodesmus* sp. and *C. vulgaris*. For nickel, in the concentration of 5.7 mg l<sup>-1</sup>, the removal efficiency reached 32% for *C. vulgaris* and 39% for *Desmodesmus* sp [47]. Mehta and Gaur [43] reported that *Chlorella vulgaris* biomass is able to remove 69% and 80% of Ni (II) and Cu (II) cations in concentrations of 2.5 ppm, respectively. While an increase in the initial concentration of Ni (II) and Cu (II) to 10 ppm, the metal removal rate was reduced only to 37 and 42%, respectively.

#### Temperature

Temperature exerts an important effect on metal speciation because most chemical reaction rates are very sensitive to temperature changes. In general, higher temperature favors greater solubility of metal ions in a solution and hence weakens the biosorption of metal ions [48]. Biosorption efficiency of each metal ion is different for each algae species with altered response to the temperature [49, 50]. Previously published studies claimed that increased algal culture temperatures could potentially increase metal ion biosorption capacity [11, 51-55]. Aksu [56] observed increased adsorption of Ni<sup>2+</sup> in the dry biomass of *C. vulgaris* with rising temperatures. Contrarily, the same author in a previous study [57] reported an increase in temperature (from 20 to 50 °C), caused a decreased cadmium (II) biosorption capacity (from 85.3 to 51.2 mg g<sup>-1</sup>). Gupta and Rastogi [11] reported lower adsorption of Cd<sup>2+</sup> by *Oedogonium* spp. at higher temperatures. Further studies suggest that metal ion uptake by some algae is exothermic and uptake capacity increases with decreasing temperature [48, 58]. But certain reports show that temperature has no effect on metal sorption [59-61, 26].

Several studies reported temperature-linked changes in metal ions uptake by living algal cells, while others also showed that the temperature has no significant influence on metal ions uptake by dead algal cells [62]. In general, different algal strains behave differently to uptake metal ions at varied temperatures [63-65].

#### Contact time

Biosorption takes place in two stages. First, for algal biomass, metal ions were passively adsorbed to cell membranes, and biosorption of metal ions occurs rapidly within the first minute. Second, for live algae, active biosorption occurs as the algal cell slowly uptakes the heavy metal ions. An increase in contact time up to the optimum contact time increases biosorption; afterward, it becomes relatively constant. The uptake of uranium (U) by biomass of nonliving *C. vulgaris* during the initial 5 min was more than 90% [66, 67]. Biomass of *Chlamydomonas reinhardtii* microalgae rapidly adsorbed free ions of Hg<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup>, with biosorption equilibrium achieved in 60 min [68, 69]. Mata *et al.*, [70] reported the amount of Au (III) adsorbed at a pH of 7 on the biomass of *Fucus vesiculosus*

macroalgae after 1 and 8 h were 28.95 mg g<sup>-1</sup> and 74.05 mg g<sup>-1</sup> of dry algae, respectively. Lamaia *et al.*, [71] measured the uptake of cadmium and lead ions by *Cladophora fracta*, separately harvested after 2, 4, 6, and 8 d, and found greater biosorption capacity was obtained in older cultures. The bio removal of heavy metal ions (Hg<sup>2+</sup>, Pb<sup>2+</sup>and Cd<sup>2+</sup>) by *Pseudochlorococcum typicum* from aqueous solution showed that the highest percentage of metal bio removal occurred in the first 30 min of contact recording 97% (Hg<sup>2+</sup>), 86% (Cd<sup>2+</sup>) and 70% (Pb<sup>2+</sup>) [14]. *Mastocarpus stellatus* attained over 50% of the total biomass cadmium uptake within 2 min of contact and over 90% in the first 9 min. Ashrutha *et al.*, [72] reported efficient removal of chromium, zinc, cadmium, lead, copper, and cobalt by bacterial consortia at approximately 75 to 85% in less than two hours of contact duration. In a study by Goher *et al.*, [73], the maximum removal efficiency for copper and lead by *C. vulgaris* at ambient room temperature was recorded at pH 5 and 20 min contact time, while the contact time of 120 min at pH 6 created the most suitable conditions for cadmium biosorption.

## CONCLUSION

The development of technology for simultaneous detection and removal of toxic metals using microalgae will assist in the bioremediation of toxic metal from contaminated sites and from industrial effluents. Understanding this mechanism will thereby enable the utilization of green, non-toxic, and efficient means of heavy metal bioremediation. This review clearly indicates that microalgae have the potential to become an effective and economical biosorbent for the bioremediation of heavy metal.

## AUTHORS CONTRIBUTIONS

All the author have contributed equally

## CONFLICT OF INTERESTS

Declared none

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